

In the Claims

Amend claim 64 as set forth below to delete the stricken-through material shown in brackets and add the underlined material.

Add the following new claims 65 through 71 inclusive.

Claims 1 – 59 (canceled)

Claim 60 (previously added and amended): A method of determining the identity of one or more nucleotide bases at specific positions of one or more nucleic acid molecules of interest, comprising:

(a) treating a sample comprising one or more nucleic acid molecules of interest, if the nucleic acid molecules of interest comprise double-stranded nucleic acid, so as to obtain unpaired nucleotide bases spanning the specific positions, or directly employing step (b) if the nucleic acid molecule of interest are single-stranded;

(b) contacting the sample from step (a) with one or more unique oligonucleotide primers,

wherein;

(i) each unique oligonucleotide primer hybridizes, under high stringency hybridization conditions, to a different stretch of nucleotide bases present in the nucleic acid molecules of interest which is immediately adjacent to the nucleotide base to be identified with that unique oligonucleotide primer, so as to form a duplex such that the nucleotide base to be identified is the first unpaired base of the nucleic acid molecule of interest immediately downstream of the 3' end of the oligonucleotide primer, and

(ii) each unique oligonucleotide primer has a unique affinity moiety which permits affinity separation of the oligonucleotide primer from all the other oligonucleotide primers and wherein the affinity moiety specifically binds to a discrete position on a solid support, such discrete position is specific for the affinity moiety of the oligonucleotide primer;

(c) contacting the duplexes from step (b), in the absence of dATP, dCTP, dGTP, or dTTP, with four different terminators, each terminator comprising a different detectable label, of a nucleic acid template-dependent primer extension reaction, wherein one of the terminators is complementary to the nucleotide base to be identified by each of the oligonucleotide primers, wherein the contacting is under conditions sufficient to permit a template-dependent primer extension reaction which incorporates the complementary terminator onto the 3' end of each of the unique oligonucleotide primers to thereby extend the 3' end of each of the unique oligonucleotide primers by one terminator;

(d) contacting the sample from step (c) with the solid support; and

(e) determining the presence and identity of the nucleotide base at the specific position or positions in each nucleic acid molecule of interest by detecting at each position of the solid support the detectable marker of the terminator incorporated at the 3' end of each of the unique oligonucleotide primers, such that each nucleotide base to be identified can be individually identified by detecting at each position of the solid support the detectable marker of the terminator incorporated at the 3' end of each unique oligonucleotide primer.

Claim 61 (previously added): A method according to claim 60, wherein the affinity moiety of each unique oligonucleotide primer comprises a sequence of nucleotides which permits attachment to the discrete positions on the solid support via base pairing of the sequence of

nucleotides to its complementary sequence of nucleotides which are attached to the solid support at the discrete positions.

Claim 62 (previously added): A method of determining the identity of one or more nucleotide bases at specific positions of one or more different nucleic acid molecules of interest, comprising:

(a) attaching a plurality of unique oligonucleotide primers to discrete positions of a solid support, wherein each unique oligonucleotide primer hybridizes, under high stringency hybridization conditions, to a different stretch of nucleotide bases present in the nucleic acid molecules of interest which is immediately adjacent to the nucleotide base to be identified with that unique oligonucleotide primer, so as to form a duplex such that the nucleotide base to be identified is the first unpaired base of the nucleic acid molecule of interest immediately downstream of the 3' end of the oligonucleotide primer;

(b) contacting the plurality of unique oligonucleotide primers of step (a) with a sample comprising a detectable amount of single stranded nucleic acid molecules of interest to form duplexes;

(c) contacting the duplexes from step (b), in the absence of dATP, dCTP, dGTP, or dTTP, with four different terminators, each terminator comprising a different detectable label, of a nucleic acid template-dependent primer extension reaction, wherein one of the terminators is complementary to the nucleotide base to be identified by each of the oligonucleotide primers, wherein the contacting is under conditions sufficient to permit a template-dependent primer extension reaction which incorporates the complementary terminator onto the 3' end of each of the unique oligonucleotide primers to thereby extend the 3' end of each of the unique oligonucleotide primers by one terminator; and

(d) determining the presence and identity of the nucleotide base at the specific position in each nucleic acid molecule of interest by detecting the detectable marker of the terminator incorporated at the 3' end of each of the unique oligonucleotide primers which have been extended.

Claim 63 (previously added and amended): A method of analyzing the sequence of nucleic acid molecules of interest, comprising:

(a) attaching a plurality of affinity moieties, wherein the affinity moiety comprises a unique sequence of nucleotides, to a solid support at defined positions;

(b) hybridizing the nucleic acid molecules of interest in solution to a plurality of oligonucleotide primers which comprise sequences of nucleotides complementary to the affinity moiety of step (a), under hybridization conditions, to generate a duplex;

(c) subjecting the hybridized primers to a template mediated single base primer extension reaction which comprises providing to the hybridized primers four terminators corresponding to each of the four nucleotide bases, to extend the hybridized primers by the addition of a terminator;

(d) sorting the extended primers by affinity capture by the affinity moieties of step (a);

(e) observing the identity and location of the terminators and thus determining the base at each of a plurality of sites of interest for the nucleic acid molecules of interest.

Claim 64 (previously added and currently amended): A method of determining the identity of one or more nucleotide bases at a plurality of specific positions in one or more nucleic acid molecules of interest, comprising:

(a) treating a sample comprising the one or more nucleic acid molecules of interest, if the nucleic acid molecules of interest comprise double-stranded nucleic acid, so as to obtain unpaired nucleotide bases spanning the specific positions, or directly employing a sample comprising the one or more nucleic acid molecules of interest in step (b) if the nucleic acid is single-stranded;

(b) contacting the sample from step (a) with a plurality of different oligonucleotide primers, wherein:

- (i) each such different oligonucleotide primer hybridizes, under high stringency hybridization conditions, to a corresponding different stretch of nucleotide bases present in the nucleic acid molecules of interest which is immediately adjacent to the specific position of a nucleotide base to be identified with that oligonucleotide primer, so as to form a duplex such that the nucleotide base to be identified is the first unpaired base of the nucleic acid molecule of interest immediately downstream of the 3' end of the oligonucleotide primer; and
- (ii) each different oligonucleotide primer [~~incorporates~~] comprises a corresponding different affinity moiety [~~which permits polymerase extension of~~], the oligonucleotide primer comprising the affinity moiety being capable of hybridizing with a nucleic-acid template and undergoing a nucleic acid template-dependent primer extension reaction with terminators of a terminator reagent [~~and permits~~], the affinity moiety permitting affinity separation of the extended oligonucleotide primer from the terminator reagent;

(c) contacting the duplexes from step (b) with a terminator reagent free of dATP, dCTP, dGTP, and dTTP and comprising four different terminators of a nucleic acid template-dependent primer extension reaction, each terminator comprising a

different detectable label corresponding to the terminator, wherein one of the terminators is complementary to a nucleotide base to be identified by each of [a] the oligonucleotide primers, wherein the contacting is carried out in a primer-extension reaction medium under conditions sufficient to permit a template-dependent primer extension reaction which incorporates the complementary terminator onto the 3' end of each of the different oligonucleotide primers to thereby extend the 3' end of each of the oligonucleotide primers by one terminator;

(d) affinity separating the respective extended oligonucleotide primers from primer-extension reaction medium by causing each of the extended oligonucleotide primers to contact an affinity group attached to a solid support, such affinity group being complementary to the affinity moiety incorporated in the oligonucleotide primer; and

(e) determining the presence and identity of the nucleotide base at each of the respective specific positions in the one or more nucleic acid molecules of interest by detecting the detectable label of the terminator incorporated at the 3' end of each of the affinity separated extended oligonucleotide primers.

Claim 65 (new): A method according to claim 64, wherein the affinity moiety of each unique oligonucleotide primer comprises a sequence of nucleotides which permits attachment to the discrete positions on the solid support via base pairing of the sequence of nucleotides to its complementary sequence of nucleotides which are attached to the solid support at the discrete positions.

Claim 66 (new): A method according to claim 64, wherein the terminators comprise dideoxynucleotides.

Claim 67 (new): A method according to claim 66, wherein the terminators comprise one